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(FILE 'HOME' ENTERED AT 11:15:21 ON 10 MAR 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 11:15:36 ON 10 MAR 2003

L1 993 S PTTG OR PTSG OR PITUITARY(3A)(TRANSFORM? OR SPECIFIC)(3A)GENE
L2 54641 S (KNOCKOUT OR NULL(3A)(MUTANT OR MUTATION))(6A)(MOUSE OR MICE
L3 2 S L1 AND L2
L4 2 DUP REM L3 (0 DUPLICATES REMOVED)

=> d bib ab 1-2 l4

L4 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS
AN 2002:815896 CAPLUS
DN 138:53529
TI Pituitary tumorigenesis in prolactin gene-disrupted mice
AU Cruz-Soto, Martha E.; Scheiber, Michael D.; Gregerson, Karen A.; Boivin, Gregory P.; Horseman, Nelson D.
CS Molecular and Cellular Physiology, University of Cincinnati College of Medicine, Cincinnati, OH, 45267, USA
SO Endocrinology (2002), 143(11), 4429-4436
CODEN: ENDOAO; ISSN: 0013-7227
PB Endocrine Society
DT Journal
LA English
AB Targeted disruption [**knockout** (KO)] of the **mouse** prolactin (PRL) gene created an animal model of primary isolated PRL deficiency in which there is no detectable PRL bioactivity. Pituitary glands of young adult female PRLKO mice were hyperplastic, and many cells had expanded cytoplasms with granular accumulations of an N-terminal peptide encoded by the disrupted PRL gene (KO/10 peptide). Confocal imaging showed that the pituitaries in PRL+/+ and PRL+/- females contained dense accumulations of apparently Golgi-assocd. immunoreactive PRL. PRLKO female mice (15-18 mo old) developed hyperemic pituitary adenomas. The pituitary tumors in PRLKO mice synthesized the KO/10 peptide, which implies that the tumors arise from the lactotroph lineage. Anchorage-independent growth was obsd. among pituitary cells from PRLKO mice, aged 8 mo or older, but not in cells from 3-mo-old PRLKO mice. GH cells appeared to be normal in PRLKO pituitaries, but were displaced by the hyperplastic and hypertrophic growth of KO/10-pos. cells. Bromocriptine suppressed mean pituitary wt. in 8-mo-old PRLKO mice compared with vehicle-treated PRLKO animals (20.+-.0.01 and 60.+-.10 mg; P < 0.01). We infer that pituitary lactotrophs of PRLKO mice suffer from a dual pathol. that includes hypertrophy resulting from endoplasmic reticulum expansion and hyperplasia, with adenomatous transformation, in part as a consequence of disrupted dopaminergic feedback regulation.
RE.CNT 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS
AN 2001:898956 CAPLUS
DN 136:211035
TI Pituitary-specific knockout of steroidogenic factor 1
AU Zhao, Liping; Bakke, Marit; Parker, Keith L.
CS Department of Internal Medicine and Pharmacology, Division of Endocrinology, University of Texas Southwestern Medical Center, Dallas, TX, 75390-8857, USA
SO Molecular and Cellular Endocrinology (2001), 185(1-2), 27-32
CODEN: MCEND6; ISSN: 0303-7207
PB Elsevier Science Ireland Ltd.
DT Journal
LA English
AB **Knockout mice** lacking the orphan nuclear receptor

steroidogenic factor 1 (SF-1) revealed its essential roles at multiple levels of endocrine development and function. These SF-1 **knockout mice** lacked adrenal glands and gonads, thereby manifesting adrenal insufficiency and sex reversal of their internal and external genitalia. Their pituitary gonadotropes failed to express several markers of normal differentiated function, and they lacked a specific hypothalamic nucleus, the ventromedial hypothalamic nucleus (VMH). Using the Cre-loxP system, the authors generated mice whose gene encoding SF-1 was inactivated specifically in the anterior pituitary. These pituitary-specific SF-1 **knockout mice** were sterile and never matured sexually. Their gonads weighed only .apprx.5% of the wt. of wild-type gonads. SF-1 immunoreactivity was absent in the anterior pituitary but was unaffected in the adrenal cortex, validating the selectivity of the gene targeting strategy. Consistent with an important role of SF-1 in gonadotropes, FSH and LH were markedly decreased in the pituitary-specific SF-1 **knockout mice**. The pituitary-specific SF-1 **knockout mice** are a novel genetic model of hypogonadotropic hypogonadism and establish essential roles of SF-1 in gonadotropin expression.

RE.CNT 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s transgen?(6a)(mouse or mice or rodent)
L5 116768 TRANSGEN?(6A)(MOUSE OR MICE OR RODENT)

=> s l1 and l5
L6 53 L1 AND L5

=> s knockout or null(3a)(mutant or mutation)
L7 82531 KNOCKOUT OR NULL(3A)(MUTANT OR MUTATION)

=> d his

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L1 993 S PTTG OR PTSG OR PITUITARY(3A)(TRANSFORM? OR SPECIFIC)(3A)GENE
L2 54641 S (KNOCKOUT OR NULL(3A)(MUTANT OR MUTATION))(6A)(MOUSE OR MICE
L3 2 S L1 AND L2
L4 2 DUP REM L3 (0 DUPLICATES REMOVED)
L5 116768 S TRANSGEN?(6A)(MOUSE OR MICE OR RODENT)
L6 53 S L1 AND L5
L7 82531 S KNOCKOUT OR NULL(3A)(MUTANT OR MUTATION)
L8 0 S L6 AND L7
L9 23 DUP REM L6 (30 DUPLICATES REMOVED)

=> d au ti so ab 1-23 l9

L9 ANSWER 1 OF 23 CAPLUS COPYRIGHT 2003 ACS
AU Gainer, H.; Yamashita, M.; Fields, R. L.; House, S. B.; Rusnak, M.
TI The magnocellular neuronal phenotype: cell-specific gene expression in the hypothalamo-neurohypophysial system
SO Progress in Brain Research (2002), 139(Vasopressin and Oxytocin: From Genes to Clinical Applications), 1-14
CODEN: PBRRA4; ISSN: 0079-6123
AB A review. The magnocellular oxytocin (OT) and vasopressin (VP) neurons of the hypothalamo-neurohypophysial system are exceptional cell biol. models to study mechanisms of cell-specific gene expression and neurosecretion of neuropeptides in the central nervous system. Single cell differential gene expression expts. have further defined these phenotypes by identifying novel and distinct regulatory mols. in these neurons. **Transgenic mouse** studies have led to the intergenic region (IGR) hypothesis, which states that the DNA sequences between the OT- and VP-genes contain crit. enhancer sites for their cell-specific expression. The recent cloning and sequencing of the human IGR, and its comparison with the mouse IGR sequence has identified conserved sequences as putative, cell-specific enhancer sites which are now being evaluated by biolistic transfections of organotypic hypothalamic cultures. With these data, it is possible to target the gene expression of specific mols. to magnocellular neurons both in vivo and in vitro, to perturb and/or visualize neurosecretory and other processes.

L9 ANSWER 2 OF 23 MEDLINE DUPLICATE 1
AU Zhou Y; Unterwald E M; Ho A; LaForge K S; Yuferov V P; Kreuter J; Sirianni M J; Allen R G; Kreek M J
TI Ablation of pituitary pro-opiomelanocortin (POMC) cells produces alterations in hypothalamic POMC mRNA levels and midbrain mu opioid receptor binding in a conditional **transgenic mouse** model.
SO JOURNAL OF NEUROENDOCRINOLOGY, (2001 Sep) 13 (9) 808-17.
Journal code: 8913461. ISSN: 0953-8194.
AB The hypothalamic-pituitary-adrenal (HPA) axis is regulated by stress-related excitatory inputs, and various inhibitory and negative-feedback controls by glucocorticoids and opioids, including pro-opiomelanocortin (POMC)-derived peptides. The role of POMC-derived peptides of pituitary origin in the modulation of brain POMC mRNA expression and opioid receptor binding was investigated using a line of **transgenic mice** that express a fusion **gene** composed of the **pituitary** expression-**specific** promoter region of the POMC gene driving the herpes simplex viral-1 thymidine kinase (TK). Male adult mice were treated with the antiherpes agent ganciclovir that selectively ablates cells expressing TK. Following treatment, POMC mRNA levels, measured by quantitative solution hybridization/RNase protection assays, were decreased by 48% in the

pituitary of the TK+/+ mice, reflecting an expected loss of the pituitary corticotrope POMC cells. This treatment also significantly lowered pituitary beta-endorphin immunoreactivity content and plasma concentrations of corticosterone. In contrast, POMC mRNA levels were increased by 79% in the hypothalamus of the TK+/+ mice with pituitary POMC cell ablation. Binding of [(3)H]DAMGO to mu opioid receptors, as measured by quantitative autoradiography, was significantly reduced in several brain regions including the central grey, median raphe and superficial grey layer of the superior colliculus. These regions are innervated by hypothalamic POMC neurones. No significant differences in binding to either kappa or delta opioid receptors were found in the brain regions studied. These results suggest that POMC-derived peptides of pituitary origin may exert a tonic negative-feedback effect on hypothalamic POMC neurones. In turn, the downregulation of central mu opioid receptors in this model may be mediated through a mechanism related to hypothalamic POMC overexpression.

L9 ANSWER 3 OF 23 SCISEARCH COPYRIGHT 2003 ISI (R)
AU Cushman L J; Camper S A (Reprint)
TI Molecular basis of pituitary dysfunction in mouse and human
SO MAMMALIAN GENOME, (JUL 2001) Vol. 12, No. 7, pp. 485-494.
Publisher: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA.
ISSN: 0938-8990.

L9 ANSWER 4 OF 23 MEDLINE DUPLICATE 2
AU Su Y; Liebhaber S A; Cooke N E
TI The human growth hormone gene cluster locus control region supports position-independent pituitary- and placenta-specific expression in the **transgenic mouse**.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Mar 17) 275 (11) 7902-9.
Journal code: 2985121R. ISSN: 0021-9258.
AB The human growth hormone (hGH) cluster contains five genes. The hGH-N gene is predominantly expressed in pituitary somatotropes, whereas the remaining four genes, the chorionic somatomammotropin genes (hCS-L, hCS-A, and hCS-B) and hGH-V, are expressed selectively in the placenta. In contrast, the mouse genome contains a single **pituitary-specific** GH **gene** and lacks any GH-related CS genes. Activation of the hGH **transgene** in the **mouse** is dependent on its linkage to a previously described locus control region (LCR) located -15 to -32 kilobases upstream of the hGH cluster. The sporadic, nonreproducible expression of hCS transgenes lacking the LCR suggests that they may be dependent on hGH LCR activity as well. To determine whether the hCS genes could be expressed with appropriate placental specificity, a series of five **transgenic mouse** lines carrying an 87-kilobase human genomic insert encompassing the majority of the hGH gene cluster and the entire contiguous LCR was established. All of the hGH cluster genes were appropriately expressed in each of these lines. High level expression of hGH was restricted to the pituitary and hCS to the labyrinthine layer of the placenta. The expression of the GH cluster genes in their respective tissues paralleled transgene copy numbers irrespective of the **transgene** insertion site in the host **mouse** genome. These studies have extended the utility of the **transgenic mouse** model for the analysis of the full spectrum of hGH gene cluster activation. Further, they support a role for the hGH LCR in placental hCS, as well as pituitary hGH gene activation, and expression.

L9 ANSWER 5 OF 23 SCISEARCH COPYRIGHT 2003 ISI (R)
AU Lee E J; Thimmapaya B; Jameson J L (Reprint)
TI Stereotactic injection of adenoviral vectors that target **gene** expression to **specific pituitary** cell types: Implications for gene therapy
SO NEUROSURGERY, (JUN 2000) Vol. 46, No. 6, pp. 1461-1468.
Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA

19106-3621.
ISSN: 0148-396X.

AB OBJECTIVE: Gene therapy is a potentially useful strategy for the treatment of pituitary adenomas or hormone deficiency disorders. We investigated the feasibility of targeting **gene** expression to **specific pituitary** cell types in vivo, using a combination of stereotactic injection and adenoviral vectors that carry pituitary-specific promoters.

METHODS: Recombinant adenoviruses containing the human growth hormone promoter (AdGHGal) or the human glycoprotein hormone alpha-subunit promoter (Ad alpha Gal) were used to drive expression of the beta-galactosidase gene. The expression of beta-galactosidase activity in the pituitary was analyzed after the administration of recombinant adenoviruses via the peripheral vein or the carotid artery, or by stereotactic injection into the rat pituitary. Double-label histology was used to evaluate cell-type expression in the pituitary.

RESULTS: Intravascular injection of AdGHGal or Ad alpha Gal failed to deliver the marker gene to the pituitary. However, direct stereotactic injection of recombinant adenoviral vectors into the pituitary achieved a high level of transgene expression. In addition, immunohistochemical staining revealed selective expression of the AdGHGal or Ad alpha Gal transgenes in pituitary cells that normally produce the respective hormones.

CONCLUSION: These findings indicate that adenoviral vectors carrying pituitary gland-specific promoters may be useful for targeted gene therapy of pituitary diseases. However, because of low transduction after peripheral administration, stereotactic injection or local administration of viruses at the time of pituitary surgery is probably required for efficient gene expression.

L9 ANSWER 6 OF 23 MEDLINE
AU Wang Z; Melmed S
TI Characterization of the murine **pituitary** tumor **transforming gene** (**PTTG**) and its promoter.
SO ENDOCRINOLOGY, (2000 Feb) 141 (2) 763-71.
Journal code: 0375040. ISSN: 0013-7227.

AB We recently isolated rat **pituitary** tumor **transforming gene** (**PTTG**) complementary DNA and showed its potent in vitro and in vivo transforming activity. We now characterize the mouse **PTTG** gene and its promoter. The entire gene is composed of five exons and four introns and spans about 7 kb. Northern analysis showed that **PTTG** was expressed in several tumor cell lines examined, but not in all normal tissues, implying a correlation between **PTTG** and tumorigenesis. Using rapid amplification of 5'-cDNA ends, the transcription start site was localized at -303 nucleotides upstream to the ATG codon in both F9 and AtT20 cells. An approximately 4.3-kb upstream region demonstrated promoter activity in AtT20 cells as well as other cell lines tested, and in vivo, the cloned promoter driving an enhanced green fluorescent protein transgene exhibited transcriptional activation in testis and embryo. Serial deletions showed that -313 bp of the 5'-flanking region was critical for promoter activity. Three elements contribute to promoter activity. Both element A (-313/-293) and element C (-180/-160), in an electrophoretic mobility shift assay using NIH-3T3 nuclear extract, formed three specific complexes, which were competed by a known Sp1 oligo; one complex was supershifted by Sp1 antibody, and the other two complexes were both supershifted by an Sp3 antibody. Two mutants disrupting element A resulted in up to 70% loss of promoter activity and abrogated formation of specific DNA-protein binding complexes, implying a more important role for element A. Element B (-249/-229) shows more than 80% homology to a consensus c-myb element, but formed two specific complexes that differed from that of c-myb in the electrophoretic mobility shift assay. Thus, the integrity and possible cooperation among these elements contribute to the basal promoter activity of the mouse **PTTG** oncogene homolog.

L9 ANSWER 7 OF 23 MEDLINE DUPLICATE 3
AU Albarracin C T; Frosch M P; Chin W W
TI The gonadotropin-releasing hormone receptor **gene** promoter directs **pituitary-specific** oncogene expression in **transgenic mice**.
SO ENDOCRINOLOGY, (1999 May) 140 (5) 2415-21.
Journal code: 0375040. ISSN: 0013-7227.
AB Our previous work has shown that 1.2 kb of the 5' flanking region of the mouse GnRH receptor (mGnRH-R) gene is sufficient to direct tissue-specific expression in vitro. In this study, we have used the cell-specific regulatory sequences of the mGnRH-R gene promoter to target the expression of the simian virus 40 virus T antigen (TAg) to the pituitary gland of **transgenic mice**. A hybrid **transgene**, GnRH-R/TAg, was prepared using the -1164/+52 region of the mGnRH-R gene and +2533/+5234 sequences encoding the large T antigen of the simian virus 40. Two founders developed tumors of apparent pituitary origin at 44 (M28, female) and 50 (M25, male) days of age. M28 and M25 mice were about 50% underweight, and their gonads were grossly underdeveloped compared with wild-type litter mates. A third male founder, M29, developed a tumor at a later time (109 days). M29 was able to breed successfully and stably transmit the GnRH-R/TAg **transgene**. **Mice** of the M29 **transgene** line developed tumors at 4-5 months of age. Gross examination showed that the tumors extend from the sella and infiltrate into the inferior surface of the brain. In small tumors collected from young transgenic animals, normal pituitary cells as well as transition areas of increasing cellular atypia are evident. Frankly malignant cells are seen in all tumors. The pituitary tumors express the alpha-, FSHbeta-, and LHbeta-subunits and the GnRH-R messenger RNA, all markers of a gonadotrope but not of other anterior pituitary cell lineages. In summary, our studies indicate that 1.2 kb of the 5'-flanking region of the mGnRH-R gene can be used to target expression specifically to the gonadotropes of the pituitary gland in **transgenic mice**. The GnRH-R gene promoter-directed expression appears to be cell-specific and results in the formation of tumors that are primarily of gonadotropic origin.

L9 ANSWER 8 OF 23 CAPLUS COPYRIGHT 2003 ACS
IN Melmed, Shlomo; Akita, Sadanori; Readhead, Carol
TI Transgenic mammals expressing a foreign leukemia inhibitory factor gene in the pituitary at high levels
SO PCT Int. Appl., 26 pp.
CODEN: PIXXD2
AB **Transgenic mice** capable of tissue-specific expression of a foreign leukemia inhibitory factor (LIF) gene at high levels in the pituitary are described. In particular, levels of expression of the gene that are .gtoreq.5-fold higher than normal are achieved using a growth hormone gene promoter. These animals are useful as animal models of pituitary disorders and are also useful for identifying compds. that stimulate growth hormone prodn., compds. useful for the treatment of physiol. disorders assocd. with craniopharyngioma, and compds. useful for the treatment of physiol. disorders assocd. with pituitary cysts, and the like. **Transgenic mice** were created by microinjection of an expression construct with a LIF cDNA under control of the rat growth hormone promoter into the pronuclei of fertilized eggs followed by implantation into pseudopregnant females. Founder mice were identified by Southern blotting. These mice were smaller than control littermates and had a shorter lifespan, could not be impregnated and showed diaphragmatic hypoplasia with chronic liver and lung congestion. The effects were not due to disruption of the endogenous LIF gene. Serum prolactin and insulin-like growth factor 1 levels in the **transgenic mice** were significantly lower than in control littermates. The **transgenic mice** showed a no. of structural changes in the pituitary, esp. affecting Rathke's cleft.

L9 ANSWER 9 OF 23 SCISEARCH COPYRIGHT 2003 ISI (R)

AU Su Y (Reprint); Liebhaber S A; Cooke N E
TI The human growth hormone locus control region supports **pituitary** - and placental-**specific** patterns of **gene** expression in **transgenic mice**.
SO FASEB JOURNAL, (31 JUL 1997) Vol. 11, No. 9, Supp. [S], pp. 1244-1244. Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998.
ISSN: 0892-6638.

L9 ANSWER 10 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AU Su, Y.; Liebhaber, S. A.; Cooke, N. E.
TI The human growth hormone locus control region supports **pituitary** - and placental-**specific** patterns of **gene** expression in **transgenic mice**.
SO FASEB Journal, (1997) Vol. 11, No. 9, pp. A1069.
Meeting Info.: 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual Meeting of the American Society for Biochemistry and Molecular Biology San Francisco, California, USA August 24-29, 1997
ISSN: 0892-6638.

L9 ANSWER 11 OF 23 CAPLUS COPYRIGHT 2003 ACS
AU Scieglinska, Dorota; Widlak, Wieslawa; Rusin, Marek; Markkula, Merja; Krawczyk, Zdzislaw
TI Expression of the testis-specific HSP70-related gene (HST70 gene) in somatic non-testicular rat tissues revealed by RT-PCR and **transgenic mice** analysis
SO Cell Biology International (1997), 21(12), 813-821
CODEN: CBIIEV; ISSN: 1065-6995
AB The hst70 gene which belongs to rat HSP70 multigene family is highly expressed in pachytene spermatocytes. Using a **transgenic mice** model it was found that the promoter of the rat hst70 gene directs the expression of the chloramphenicol acetyl transferase (CAT) reporter gene not only to testis but also to multiple somatic tissues. In wild-type rats the expression of the hst70 gene in tissues other than testis was confirmed by non-quant. reverse transcription polymerase chain reaction (RT-PCR) anal. Beside the testis, the CAT expression in **transgenic mice** and the hst70 gene transcripts in wild-type rats were found in brain, pituitary, epididymis, vas deferens, adrenals, spleen, lung, ovary, oviduct and uterus. The only tissue in which both the CAT expression and the hst70 gene activity has not been found was the liver. These observations suggest possibly more universal, not confined to spermatogenesis, function of the hst70 gene. (c) 1997 Academic Press.

L9 ANSWER 12 OF 23 SCISEARCH COPYRIGHT 2003 ISI (R)
AU SZETO D P (Reprint); RYAN A K; OCONNELL S M; ROSENFELD M G
TI P-OTX - A PIT-1-INTERACTING HOMEODOMAIN FACTOR EXPRESSED DURING ANTERIOR-PITUITARY GLAND DEVELOPMENT
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (23 JUL 1996) Vol. 93, No. 15, pp. 7706-7710.
ISSN: 0027-8424.
AB A novel OTX-related homeodomain transcription factor has been identified on the basis of its ability to interact with the transactivation domain of the pituitary-specific POU domain protein, Pit-1, This factor, referred to as P-OTX (pituitary OTX-retated factor), is expressed in primordial Rathke's pouch, oral epithelium, first branchial arch, duodenum, and hindlimb. In the developing anterior pituitary, it is expressed in all regions from which cells with distinct phenotypes will emerge in the mature gland. P-OTX is able to independently activate and to synergize with Pit-1 on **pituitary-specific** target **gene** promoters, Therefore, P-OTX may subserve functions in generating both precursor and specific cell phenotypes in the anterior pituitary gland and in several other organs.

L9 ANSWER 13 OF 23 MEDLINE DUPLICATE 4
AU Jones B K; Monks B R; Liebhaber S A; Cooke N E
TI The human growth hormone gene is regulated by a multicomponent locus control region.
SO MOLECULAR AND CELLULAR BIOLOGY, (1995 Dec) 15 (12) 7010-21.
Journal code: 8109087. ISSN: 0270-7306.
AB The five-member human growth hormone (hGH)/chorionic somatomammotropin (hCS) **gene** cluster encodes the **pituitary-specific** hGH-N **gene** and four highly related genes (hGH-V, hCS-A, hCS-B, and hCS-L) that are expressed only in the placenta. When the hGH-N or hCS-A gene, together with all previously identified cis-acting regulatory sequences, was integrated into the mouse genome, it was expressed only sporadically and at low levels in the transgenic target organs. DNase I mapping of chromatin from expressing and nonexpressing cell types was used to identify a pituitary-specific set of DNase I-hypersensitive sites (HS) and a set of HS common to both the pituitary and placenta, centered approximately 15 and 30 kb 5' of hGH-N, respectively. When contained on a cosmid insert in their native genomic configuration, these HS consistently directed high-level, pituitary-specific expression of hGH-N in **transgenic mice** and appeared to define a locus control region required for hGH-N expression. Individually, each set of HS was able to mediate position-independent hGH-N expression in the pituitary but demonstrated loss of physiologic control and loss of tissue specificity. The gene-proximal set of HS contained a potent enhancer activity in the pituitary, while the more distal set appeared to function primarily to establish site-of-integration independence. These data indicate that synergistic interactions among multiple elements are required to restrict hGH-N transcription to the pituitary and generate appropriate levels of expression. In addition, these results suggest a role for both shared and unique regulatory sequences in locus control region-mediated expression of the hGH/hCS gene cluster in the pituitary and possibly the placenta.

L9 ANSWER 14 OF 23 MEDLINE DUPLICATE 5
AU Allen R G; Carey C; Parker J D; Mortrud M T; Mellon S H; Low M J
TI Targeted ablation of pituitary pre-proopiomelanocortin cells by herpes simplex virus-1 thymidine kinase differentially regulates mRNAs encoding the adrenocorticotropin receptor and aldosterone synthase in the mouse adrenal gland.
SO MOLECULAR ENDOCRINOLOGY, (1995 Aug) 9 (8) 1005-16.
Journal code: 8801431. ISSN: 0888-8809.
AB We have produced and characterized lines of **transgenic mice** expressing a fusion **gene** composed of the **pituitary** expression-**specific** promoter region of the POMC gene, driving the herpes simplex viral-1 thymidine kinase. Adult mice were treated with the antiherpes agent ganciclovir at 70 mg/kg body weight (ip, twice daily for 10-12 days). Approximately 98% of the pituitary intermediate lobe melanotropes and anterior lobe corticotropes were ablated as determined by immunocytochemistry and RIA specific for the POMC-derived peptides, ACTH, beta-endorophin, and alpha-MSH. The number of lactotropes, somatotropes, thyrotropes, and gonadotropes was not altered compared with controls, indicating that in the adult pituitary, POMC products are not required to maintain the distribution of cell types. As expected, plasma corticosterone levels were substantially decreased after POMC cell ablation. In situ hybridization studies showed that the mouse ACTH receptor was expressed uniformly throughout the adrenal cortex, and RNase protection assays revealed that the ACTH receptor mRNA decreased after pituitary POMC cell ablation. Additionally, RNase protection assays showed that pituitary POMC cell ablation resulted in the decrease of adrenal p450c11 beta transcripts while p450c11AS (aldosterone synthase) mRNA levels remained constant. These data demonstrate differential regulation of steroid pathway-specific enzymes by POMC products. Our results also suggest that the thymidine kinase cell obliteration technique

may not be dependent on cell division as a prerequisite for cytotoxicity, thus supporting the idea that targeted molecular ablation using cell- and tissue-specific promoter sequences to drive viral thymidine kinase expression can be refined further to study other nonmitotic cells.

L9 ANSWER 15 OF 23 MEDLINE DUPLICATE 6
AU Liu B; Mortrud M; Low M J
TI DNA elements with AT-rich core sequences direct pituitary cell-specific expression of the pro-opiomelanocortin gene in **transgenic mice**.
SO BIOCHEMICAL JOURNAL, (1995 Dec 15) 312 (Pt 3) 827-32.
Journal code: 2984726R. ISSN: 0264-6021.
AB Corticotrophs are the first fully differentiated cells to appear in the anterior pituitary during organogenesis and are distinguished by pro-opiomelanocortin (POMC) gene expression. Earlier studies in our laboratory defined three DNA regions (sites 1, 2 and 3) within promoter sequences at the 5'-end of the rat POMC gene (-323/-34) that cooperatively targeted cell-specific gene expression to corticotrophs and melanotrophs in **transgenic mice**. In this study we analysed the DNA-nuclear protein interactions underlying this functional activity. We demonstrated that the transcriptional activator SP1 interacts with GC-rich regions in sites 1 (-146/-136) and 2 (-201/-192) and an unidentified protein, which we call PP1 (putative pituitary POMC1), interacts with AT-rich regions in sites 2 (-202/-193) and 3 (-262/-253). The PP1-binding activity appears to be specific to cells that express the POMC gene because it was detected in nuclear extracts prepared from AtT20 corticotroph cells and mouse melanotroph tumours but not from GH4 pituitary tumour cells, HeLa cells or liver. Site-directed mutagenesis of core binding sequences demonstrated that PP1 is required for the correct cell-specific expression of the POMC gene in the pituitary gland of **transgenic mice** and SP1 appears to support such an expression. The best core binding sequence for PP1 is TAAT, a possible transcription factor homeodomain contact site. However, PP1 is distinct from Brn 3.0, a POU protein that also binds to site 3. We conclude that PP1 is a transcriptional activator for **pituitary-specific** POMC **gene** expression.

L9 ANSWER 16 OF 23 MEDLINE DUPLICATE 7
AU Lipkin S M; Naar A M; Kalla K A; Sack R A; Rosenfeld M G
TI Identification of a novel zinc finger protein binding a conserved element critical for Pit-1-dependent growth hormone gene expression.
SO GENES AND DEVELOPMENT, (1993 Sep) 7 (9) 1674-87.
Journal code: 8711660. ISSN: 0890-9369.
AB The growth hormone (GH) and prolactin **genes** require the **pituitary-specific** POU domain transcription factor Pit-1 for their activation. However, additional factors are necessary for the effective expression of these genes. Analysis of evolutionarily conserved sequences in the proximal GH promoter suggests the critical importance of one highly conserved element located between the two Pit-1 response elements. Mutation of this site decreases expression of a **transgene** in **mice** > 100-fold. We have identified a major activity binding to this site as a novel member of the Cys/His zinc finger superfamily, referred to as Zn-15. The Zn-15 DNA-binding domain comprises three zinc fingers separated by unusually long linker sequences that would be expected to interrupt specific DNA site recognition. Zn-15 synergizes with Pit-1 to activate the GH promoter in heterologous cell lines in which this promoter is only minimally responsive to Pit-1 alone. Our data suggest that functional interactions between the tissue-specific POU domain factor Pit-1 and this novel zinc finger factor binding to an evolutionarily conserved region in the GH promoter may constitute an important component of the combinatorial code that underlies the effective expression of the GH gene.

L9 ANSWER 17 OF 23 CAPLUS

AU Low, M. J.; Liu, B.
TI Proopiomelanocortin gene expression in **transgenic mice**
SO International Congress, Symposium and Seminar Series (1993), Volume Date 1992, 3(PROGRESS IN ENDOCRINOLOGY), 519-22
CODEN: ICGSEM; ISSN: 0969-2622
AB Fusion genes were constructed using promoter sequences derived from the 5' flanking region of the rat proopiomelanocortin (POMC) gene and either Escherichia coli .beta.-galactosidase or the K1 non-transforming mutant SV40 T antigen as reporters of gene expression. These reporter proteins were chosen because of the availability of immunohistochem. and in situ hybridization methods to detect their co-expression with the endogenous POMC gene in individual cells at the histol. level. **Transgenic mice** were produced by the pronuclear microinjection of DNA in fertilized oocytes and the transgenic founders were outbred to produce pedigrees for the anal. of reporter gene expression. POMC regulatory elements sufficient for accurate **pituitary-specific gene** transcription were located between nucleotide positions -323 and +64. The consistent lack of arcuate neuronal expression even with the 4 kb construction suggests that a neural-specific element is contained elsewhere in the gene. Transgene expression was first detected in intermediate lobe melanotrophs at day E15.5 confirming that the correct spatial and temporal activation of POMC gene expression utilizes the identical minimal regulatory elements required for cell-specific and hormonal regulation. DNase I protection assays identified three footprinted elements in the POMC promoter; -1600 to -107 (site 1), -218 to -182 (site 2), and -281 to -249 (site 3). Synthetic oligonucleotides corresponding to these three sites each bound specifically to multiple AtT20 nuclear ext. factors in gel-shift assays. Southwestern assays demonstrated that a 60 kDa protein bound to each of the three POMC promoter sites in addn. to an SP-1 transcription factor consensus binding site oligonucleotide; however, neither site 2 nor 3 oligonucleotides competed for radiolabeled site 1 binding. These data suggest that a protein of 60 kDa binds to each of these sites with different affinities, possibly because of a conserved GC-rich motif, and may be a part of the machinery responsible for accurate transcriptional regulation of the POMC gene. To test the functional role of the three protected sites found in the POMC promoter between nucleotides -323 and -34, the authors introduced a final series of **transgenes** into **mice**. Individual block deletions of these three sites did not alter the pituitary-specific or hormonally regulated transgene expression. However, the combined deletion of sites 2 and 3 resulted in a complete loss of transgene expression. It is concluded from these data that the three footprinted sites are functionally interchangeable and act in combination with promoter elements between -114 and -34 as the minimal regulatory elements in the POMC gene that are both necessary and sufficient for the correct spatial, temporal and hormonally regulated expression of the POMC gene in the pituitary gland.

L9 ANSWER 18 OF 23 MEDLINE DUPLICATE 8
AU Rubinstein M; Mortrud M; Liu B; Low M J
TI Rat and mouse proopiomelanocortin **gene** sequences target tissue-**specific** expression to the **pituitary** gland but not to the hypothalamus of **transgenic mice**.
SO NEUROENDOCRINOLOGY, (1993 Oct) 58 (4) 373-80.
Journal code: 0035665. ISSN: 0028-3835.
AB The proopiomelanocortin (POMC) gene is expressed predominantly in corticotrophs of the pituitary anterior lobe, melanotrophs of the intermediate lobe and neurons of the arcuate nucleus of the hypothalamus. The different ontogeny of POMC mRNA as well as the complicated hormonal regulation of POMC gene expression in the three different cell types suggests a concerted interaction between several cis-acting elements in the POMC gene and transcription factors located in each of the three cell types. To investigate cell-specific elements in the POMC gene we tested two different constructs in **transgenic mice**. The

construct -4000rPOMCLacZ, carrying 4 kb of the rat POMC promoter fused to the Escherichia coli beta-galactosidase gene, showed appropriate expression in melanotrophs in 50% of the mice analyzed. beta-Galactosidase activity was less evident in corticotrophs under basal environmental conditions. In brain, 7 out of 15 independently derived transgenic founders had ectopic expression of the transgene in different areas; however, none of the animals analyzed expressed beta-galactosidase in neurons of the arcuate nucleus. The construct HAL*, a 'tagged' 10.2-kb mouse genomic fragment, was more efficiently targeted to the pituitary. Using in situ hybridization, we detected uniform expression of HAL* in melanotrophs in 100% of the 6 pedigrees analyzed and transgenic mRNA levels paralleled those of the endogenous POMC mRNA. In corticotrophs, basal expression was low but after adrenalectomy HAL* mRNA levels were comparable to those of POMC. None of the 6 pedigrees had appropriate expression of HAL* in the brain; however, 2 lines had ectopic expression in the dentate gyrus of the hippocampus. (ABSTRACT TRUNCATED AT 250 WORDS)

L9 ANSWER 19 OF 23 CAPLUS COPYRIGHT 2003 ACS
AU Stefaneanu, Lucia; Rindi, Guido; Horvath, Eva; Murphy, David; Polak, Julia M.; Kovacs, Kalman
TI Morphology of adenohypophysial tumors in **mice transgenic** for vasopressin-SV40 hybrid oncogene
SO Endocrinology (1992), 130(4), 1789-95
CODEN: ENDOAO; ISSN: 0013-7227
AB **Transgenic mice** for the promoter sequence of bovine arginine vasopressin (AVP) gene fused to large SV-40 T-antigen coding sequence develop pituitary tumors and insulin-producing pancreatic tumors. To establish the cellular compn. of the pituitary tumors, histol., immunocytochem., in situ hybridization, and electron microscopic techniques were applied. Pituitary anterior lobe tumors were identified in 10 out of 14 glands examd. In 2 cases, intermediate lobe tumors were also found. The anterior lobe tumors contained a variable no. of growth hormone immunoreactive cells. Hybridization performed in 7 cases revealed a diffuse distribution of growth hormone mRNA over all tumor cells. Ultrastructurally, the tumors contained undifferentiated cells with very small secretory granules and rare cells showing some resemblance to somatotrophs. The presence of a few prolactin (PRL) immunoreactive cells in 4 tumors and scattered TSH immunoreactive cells in 2 tumors supports the view that somatotrophs have the potential to produce PRL and TSH. The intermediate lobe tumors were immunoreactive for ACTH and intensely pos. for proopiomelanocortin mRNA. In the nontumorous adenohypophyses, no hyperplasia of any cell type was noted. Several growth hormone immunoreactive cells exhibited pleomorphic giant nuclei and mitoses. Thus, the majority of **transgenic mice** for AVP/large T-antigen develop pituitary tumors originating in and composed of somatotrophs. Less frequently, intermediary lobe tumors were present as well. AVP/SV40 **transgenic mice** provide a unique model for somatotroph tumors that are neither preceded by, nor assocd. with somatotroph hyperplasia.

L9 ANSWER 20 OF 23 MEDLINE DUPLICATE 9
AU Keri R A; Andersen B; Kennedy G C; Hamernik D L; Clay C M; Brace A D; Nett T M; Notides A C; Nilson J H
TI Estradiol inhibits transcription of the human glycoprotein hormone alpha-subunit gene despite the absence of a high affinity binding site for estrogen receptor.
SO MOLECULAR ENDOCRINOLOGY, (1991 May) 5 (5) 725-33.
Journal code: 8801431. ISSN: 0888-8809.
AB Chronic administration of estradiol inhibits transcription of the gene encoding the alpha-subunit of pituitary glycoprotein hormones. Here, we show, using transfection analyses and a filter binding assay, that 1500 basepairs of proximal 5' flanking sequence of the human alpha-subunit gene lack a functional estrogen response element when transfected into heterologous cell lines, and fail to bind estrogen receptor purified from

calf uterus. Yet, this same region of the alpha-subunit gene confers estradiol responsiveness (transcriptional suppression) to the bacterial chloramphenicol acetyltransferase gene in **transgenic mice**. A smaller promoter fragment of the bovine alpha-subunit gene also confers responsiveness to estradiol in **transgenic mice**, suggesting that the same element may mediate the steroid responsiveness of both promoters. Furthermore, regulation by estradiol of the chimeric human or bovine alpha-chloramphenicol acetyltransferase **genes** is **pituitary specific**, underscoring the physiological significance of these studies. Based on these results, we conclude that estradiol regulates expression of the alpha-subunit gene in vivo through a mechanism that does not involve high affinity binding of estrogen receptor to the alpha-subunit gene. Whether this mechanism is manifest at the level of the pituitary or hypothalamus remains to be determined.

L9 ANSWER 21 OF 23 MEDLINE DUPLICATE 10
AU Hammer G D; Fairchild-Huntress V; Low M J
TI **Pituitary-specific** and hormonally regulated **gene** expression directed by the rat proopiomelanocortin promoter in **transgenic mice**.
SO MOLECULAR ENDOCRINOLOGY, (1990 Nov) 4 (11) 1689-97.
Journal code: 8801431. ISSN: 0888-8809.
AB All aspects of POMC biosynthesis exhibit tissue-specific regulation. The single copy gene is highly expressed in anterior lobe (AL) corticotrophs and intermediate lobe (IL) melanotrophs of the pituitary gland and in the arcuate nucleus of the hypothalamus. POMC gene transcription in corticotrophs is induced by hypothalamic CRH and vasopressin and inhibited by adrenal glucocorticoids, while in melanotrophs it is predominantly regulated by beta-adrenergic neural input and dopamine. To identify the rat POMC (rPOMC) gene sequences necessary and sufficient to target expression and hormonal regulation in corticotrophs and melanotrophs, we generated 13 **transgenic mice** carrying rPOMC fusion genes. The genes consisted of 706 or 480 basepairs of rPOMC 5' flanking sequences ligated to either the E. coli LacZ gene encoding beta-galactosidase or the K1 mutant of the SV40 large T-antigen gene. Overall, half of the transgenic lines had reporter gene expression in their AL and IL in a pattern indistinguishable from ACTH immunohistochemistry. In three of these lines, beta-galactosidase or K1 T-antigen was localized by double immunofluorescence exclusively to ACTH-positive corticotrophs and melanotrophs. Transcriptional regulation of the rPOMC-LacZ fusion gene in response to hormonal manipulation was quantified by a fluorescence assay for beta-galactosidase enzyme activity in pituitary extracts. There was a 15-fold increase in AL enzyme activity after adrenalectomy and a 3-fold increase in IL activity after haloperidol treatment. X-gal histochemistry of pituitaries from hormonally treated mice confirmed the cellular specificity of these effects.(ABSTRACT TRUNCATED AT 250 WORDS)

L9 ANSWER 22 OF 23 CAPLUS COPYRIGHT 2003 ACS
AU Tremblay, Yves; Tretjakoff, Irene; Peterson, Alan; Antakly, Tony; Zhang, Cun Xian; Drouin, Jacques
TI Pituitary-specific expression and glucocorticoid regulation of a proopiomelanocortin fusion gene in **transgenic mice**
SO Proceedings of the National Academy of Sciences of the United States of America (1988), 85(23), 8890-4
CODEN: PNASA6; ISSN: 0027-8424
AB The product of a single gene encoding proopiomelanocortin (POMC) is differentially processed to produce ACTH and .alpha.-melanotropin in anterior and intermediate pituitary cells, resp. Hormonal control of POMC gene transcription and of ACTH or .alpha.-melanotropin release is also tissue-specific; for example, glucocorticoids specifically inhibit anterior but not intermediate pituitary POMC transcription. Outside the pituitary gland, very low levels of POMC mRNAs are present in brain,

testes, ovaries, and placenta. **Transgenic mice** were used to identify POMC 5' flanking sequences that are sufficient for tissue-specific expression and glucocorticoid regulation in anterior and intermediate pituitary cells. Three lines of **transgenic mice** were established, each carrying 50-75 copies (per cell) of a chimeric rPOMCneo gene constituted of rat POMC promoter sequences and of bacterial neomycin-resistance coding sequence. High levels of rPOMCneo transcripts were detected in pituitaries of mice from all 3 lineages. In situ hybridization revealed that the ratio of intermediate to anterior pituitary transcripts was similar for the transgene and endogenous POMC mRNA. The rPOMCneo transcripts were not detected in any other tissue except at very low levels in the testes in 2 transgenic lines. Endogenous mouse POMC mRNA increased in response to depletion of plasma glucocorticoids (adrenalectomy) and decreased after glucocorticoid treatment; rPOMCneo transcripts were altered to the same extent by these treatments in all three lines. Intermediate pituitary and testicular rPOMCneo transgene expression was not altered by these treatments. Thus, no more than 769 base pairs of the rat POMC promoter are required for pituitary-specific expression and for specific glucocorticoid inhibition of the POMC gene in the anterior pituitary.

L9 ANSWER 23 OF 23 CAPLUS COPYRIGHT 2003 ACS

AU Lira, Sergio A.; Crenshaw, E. Bryan, III; Glass, Christopher K.; Swanson, Larry W.; Rosenfeld, Michael G.

TI Identification of rat growth hormone genomic sequences targeting pituitary expression in **transgenic mice**

SO Proceedings of the National Academy of Sciences of the United States of America (1988), 85(13), 4755-9
CODEN: PNASA6; ISSN: 0027-8424

AB Constructs contg. different segments of the 5' flanking region of the rat growth hormone gene fused to the human growth hormone coding sequences were introduced into fertilized mouse oocytes. As few as 181 base pairs of the rat growth hormone promoter targeted reporter gene expression to the pituitary gland of the resulting **transgenic mice**.
A construct contg. only 45 base pairs of the promoter failed to target expression of the reporter to the pituitary, indicating that the pituitary expression is directed by information contained in the segment spanning positions -181 to -45. In the pituitary, immunohistochem. showed transgene expression mainly in the growth hormone-producing cells (somatotrophs), in a subset of cells producing TSH, and occasionally in prolactin-producing cells. These data establish that cis-active elements contained within the first 180 base pairs of the promoter are sufficient for transcriptional activation of the growth hormone gene in somatotrophs and suggest a functional relationship among growth hormone, prolactin, and TSH gene activation.